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THE HISTORY OF BACTERIA INHALATION

Bacteria and viruses are aerosols because, due to
their size, they act as suspended matter.

Following century-long speculations concerning the
origin of infectious diseases, the observation of the small-
est organisms related to disease became increasingly more
frequent after the invention of the microscope. The actual
relationships between irritant and disease were not recog-
nized until the period of classical bacteriology, most out-
standingly represented by L. Pasteur and R. Koch. This pe-
riod is briefly characterized in the first part of this
chapter. The second part deals in detail with experiments
of bacteria inhaled by animals. These were performed in the
late nineteenth and early twentieth century, and concentrat-
ed primarily on the tubercular bacillus.

Spectulations Concerning the Lung as Entrance for Infection Agents

In the search for the etiology of contagious diseases,
the miasma, which floats in the surrounding atmosphere and
causes putrefaction in the body through inhalation, played
an important role as a "pathological secretion". Galen
(129-210) ascribed this infectious putrefaction of the air
to unburied corpses (war epidemic), swamp exhalations, and
excessive heat. Later, various putrescent processes were

added to the list, and pathological exhalations from the interior of the earth were assumed.

In the course of centuries, the concept of contagion was added as opposed to that of miasma. After a var: of concepts, Pettenkofer in the second half of the . th century arrived at the following definition: i.. diseases are miasmatic when the toxin enters the or... from the outside, regardless of its origin; however, it must mature outside the organism before it can become infectious. Contagious diseases, on the other hand, are com-nicated by direct contact from body to body, or through... inoculation of infected objects, without the infect.. having to undergo changes.

During the years, various theories prevailed concerning the origin and nature of the pathological agent. Early authors regarded the miasma as a poisonous gas floating in the atmosphere, forcing its way into the body with air and food, and through the pores, and causing putrescence of the humours and the spiritus vitalis. The nature of contagion was explained by means of comparison; communication from one body to another was compared to the spreading of putres-cence from one apple to another. The observation that the slightest trace of contagious sufficed to infect the entire organism was compared to the old experience that a small amount of sour dough would set a large amount of another dought in fermentation. The many views concerning the agent crystallized into three theories which prevailed until the beginnings of modern bacteriology: poisons, ferments, or live organisms.

With the invention of the micrscope (1590 by Jan..en), an unsuspected new world of tiny organisms became accessible to the eye. The idea of a contagium vivum became increasingly more plausible, and shortly afterwards a complete pathologia animata was developed. The Jesuit scholar ...

... victims. He does not describe these worms ... ever, the organisms he saw in the lymphs were ...

In 1675, Leeuwenhoeck was the first to describe ... to depict bacteria. He found "animacula" (bact...... diarrhetic feces; however, he excluded the possibility of...

their penetrating into the blood, because it seemed to him that the ... ae fissures were too small. Initially he h.. ... "the most minute animalcules" in plant infusion.. ...d 1876, L. ..rmull.. and Wrisberg named these infusor.. ... hey could be found in plant infusions. O.F. Mull.. ....nd 1780 to classify infusoria, which, at th.. ..., include.. bacteria as well. In 1838, Chr. Ehrenberg wrote a systematic dissertation about infusoria.

Henle (1840) decisively spoke out for the existence of a contagium vivum, at least in the case of miasmatic-contagious and purely contagious diseases. He was fully aware of the conditions which had to be fulfilled before a para-site could be considered the stimulant of a disease: constant proof, isolation, and testing of isolated organisms. L. Pasteurs experiments on fermentation had a considerable influence on the theory concerning the contagium vivum. In a heated discussion with Liebig, Pasteur was able to prove that the process of fermentation is conditioned by microbes rather than by decaying nitrogen substances. Putrefaction, too, was no other process than the decomposition of organic matter caused by microorganisms.

Pasteur's belief that every type of fermentation is caused by a specific microorganism brought us closer to the idea that a specific microorganism would have to represent the stimulant. It was difficult to classify the multitude of bacteria -- particularly the widely-described stimulants of wound infections, to separate them morphologically and biologically, and to relate them to disease. The subsequent development led to the question whether well-characterized and constant types of bacteria actually did exist, or whether, in the end, there was only one type of highly variable bacteria. The solution was the existence of specific types of bacteria, proved by Robert Koch in his fundamental works. In his first dissertation on anthrax, which appeared in 1876, he discussed the development of the anthrax bacillus after having followed it under the microscope from spore to spore, and proved that anthrax in animals was produced only by injecting the anthrax bacillus or one of its spores, but not one of the other bacteria.

Important aids in the research of pathogenic microorganisms were the production of suitable culture media for bacteria in vitro, an improved representation of bacteria through aniline dyes, and the use of a condensor (Abbe) in illuminating microscopes. This research reached its peak in 1882 when Robert Koch discovered the tubercular bacillus.

The fact that air contains living organisms was first proved by Ehrenberg in 1838, and two years later by Gaultier de Glaubry. At that time, however, little attention was paid to these discoveries. It was Pasteur (1822-1895) who managed to focus general interest on the importance of germs in the atmosphere. Robert Koch (1831) successfully used the sedimentation method to develop pathogenic germs from the air in prepared media and to differentiate them extensively.

Chour, Favier, Olivier, Fereol, Squire, Vernet, Kuhl, and others continued to investigate infection through the open air in typoid. Romano even stored a bouillon culture mixed with dust in an ordinary room. The experiment negated the possibility of air infection by typhoid bacilli, since the latter cannot survive beyond the degree of dryness necessary to make the dust float in the atmosphere and become inhalable.

Abel, Loffler, Park, Germano, and others published their observations of air infection in diphtheria, and Chatin, Solovyev, Zielienyev, Germano, and others published observations about aerogenous infection with streptococci. Other scientists reported on the communication of chloera, pestilence, and cerebro-spinal meningitis through the air. Other contributions of that period are discussed in detail in the excellent summery by E. Germano, "The Communication of Infectious Diseases through the Air," Zeitschrift Hygiene (Periodical for Hygiene), No 24, 25, 26, 1897.

Inhalation Experiments and Tubercular Bacilli

The chief forerunners of this era were Schottelius and Tappeiner, who published their findings around the turn of the century, that is before the tubercular bacillus was discovered.

Schottelius (1876), whose experiments regarding the effect of inhaled substances had been discussed in "The History of Dust Inhalation," (9, 3/4), experimented with the inhalation of organic dust particles which can be decomposed in the lung in addition to the unorganic substances whose effect is purely "mechanical". He used the sputum of several tubercular phthisis patients, as well as of persons whose diagnosis excluded the possibility of lung tuberculosis. Other substances used were Limburger cheese and very fresh, still warm brain of calves, pigs, and bulls.

The sputum used for inhalation thin liquid mucus, and was diluted with water until the filtered solution

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obtained a milky white color and a somewhat oily consistency.
The method was as follows: the animal was placed in a wood-
en box which corresponded to its size. Iron bars divided
the box into a small front chamber. A collar and chain held
its head toward the front. The walls of the back chamber
were equipped with large air holes. Two similar opening
were to be found in the front chamber next to the entrance
of the speculum. The funnel-like end of the speculum out-
side the front chamber of the inhalation booth was connect-
ed a magnified Siegel inhalator which reduced the inhaled
matter to dust. A thermometer in the front chamber control-
led the temperature. The duration of the experiment usually
lasted for several weeks, with a daily one-hour inhalation
period.

Following the inhalation of various substances,
Schottelius found small nodules in the lung. Macroscopical-
ly they gave the impression of miliary tubercles, while his-
tologically and etiologically they had a different meaning.
He writes: "These knots apparently are primary inflammation
sources caused by the direct irritation of the alveolar wall
through substances entering with the stream of air, conse-
quently foreign body pneumoniae in the most subtle sense
of the word. I do not dare to offer definite statements
pertaining to such questions as whether all these irritated
alveoli and alveolar groups reach the more intensive stages
of superficial inflammation, or return to normalcy follow-
ing a light catarrh, or whether perhaps the degree of inflam-
mation depends on the quantity of irritation in these cases.
Another group of nodules to be found is secondary in nature,
caused by the irritation of points usually located in the
adventitious system of lymphatic vessels. There is no doubt,
however, that these parts are irritated by inflammation
stimulants brought in by the alveoli. (Cf. the effect of
unorganic types of dust). The origin of the third group
of nodules results from the closure of small bronchi. These
are the least numerous; however, a disection of the lung
makes them accessible to the human eye."

Schottelius did not regard these results as a solu-
tion to the specific problem of tuberculosis, because non-
tuberculous, indifferent sputum causes changes similar to
the inhalation of tubercular sputum. Nevertheless, his in-
halation experiments were a step towards the assumption that
under given circumstances lung consumption might be acquired
through self-infection (effected by decomposed bronchial se-
cretion) in loco, or through inhalation and aspiration.

Tappeiner (1,2) was convinced that tuberculosis is
communicated through the inhalation of consumptive sputum

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which is emitted through coughing and remains suspended in
the air. He conducted several experiments on dogs in the
Munich Pathological Institute (Prof. Dr. V. Buhl) in the
summer of 1877, and in Meran in 1878.

The matter inhaled consisted of one tea- or table-
spoon of tubercular sputum mixed with 300-500 ccm water to
produce an emulsive, relatively clear fluid. The dissolu-
tion was done by means of a steam pulverizer which was
placed outside the testing enclosure. The animals (dogs)
were accomodated in a wooden crate, one side of which was
left open and could be closed by a latticed screen. This
opening was covered with an oilcloth during inhalation.
A hole in the cloth admitted the steam jet into the test
room. In the first series of experiments, the dogs inhaled
daily for a period of one hour, spending the rest of the
time in the test room. In the second series, they inhaled
once daily and spent the rest of the time in the open air.
The third series took place in a roughly constructed $12m^3$
shed which was placed outside. Very small amounts of sputum
were given. The testing period lasted up to two months.

Tappeiner (1) found pronounced miliary tuberculosis
in both lungs; it was not as marked or regular in the kid-
neys, and in isolated appeared during the third week fol-
lowing the first inhalation day. A small amount of pulver-
ized sputum was needed to produce these nodules. In addi-
tion, he found that the sputum particles which reach the
lunbs act in a specific way, rather than as mere foreign
matter. In addition to assuming that relatively large quan-
tities of the sputum particles inhaled go directly into the
alveoli and condition the infection, Tappeiner discussed a
second mode of infection, namely, through the intestine.
Some of the inhaled sputum particles, which settle on the
lining of the throat and pharynx, are swallowed and are re-
absorbed from the intestine. Experiments, however, which
dealt with feeding tubercular sputum to the animals showed
negative results.

Basing his views on the positive results of the in-
halation tests on dogs, Tappeiner saw great probability in
the communication of tuberculosis from man to man though
phthisic sputum which had been coughed up and reduced to
dust, particularly in poorly ventilated rooms.

In one of his later works (1880), Tappeiner (2) made
new experimental contributions to inhalation tuberculosis
in dogs. In repeating his earlier experiments with inhaled
phthisic sputum, he found that lung tuberculosis could be

produced with or without a general tubercular infection.
Inhalations of croupous and caseous lymphatic gland pus
and bronchial-purulent sputum, however, proved to be nega-
tive.

As early as 1884, Robert Koch maintained that it was
possible to communicate tuberculosis among humans through
inhalation of both sputum particles in the air and dry phthi-
sic sputum. He regarded the latter as the most likely one
to cause the infection.

G. Cornet, a student of R. Koch, extensively inves-
tigated the spreading of the tubercular bacillus outside the
organism, that is, tubercular germs which float freely in
the air or are deposited in the dust.

In order to determine the degree of infectiousness,
he collected dust which had settled on walls, bed linen,
door frames, floors, carpets, clothing, and so forth, mixed
some of it with dry buillon culture, and deposited it in the
stomach cavity of guinea pigs. The dust came from hospitals,
mental institutions, phthisis divisions in hospitals for in-
curables, private apartments, and hotel rooms of tubercular
patients. He was unable to prove the previously assumed
ubiquity of the tubercular bacillus. He also thought it
impossible that the evaporation of fluid containing bacter-
ia or a breath of air could spread bacillus germs in the
surrounding area. In Cornet's opinion, it was a firm fact
that the exhalation of consumptive patients could not pos-
sibly contain tubercular bacilli, nor did their sputum --
provided it remained damp -- pass bacilli into the air. Small
particles containing bacilli might reach the air, however,
only if the cought was particularly bad. This danger was
not great, because such explosive cough usually caused the
loss of saliva from the teeth, palate, and lips. In most
cases, this saliva does not contain bacilli. Most frequent-
ly bacilli get into the air after the secretion containing
bacilli dries out, and individual particles of this dry mass
are separated through mechanical influences. The difficulty
in pulverizing and reducing sputum to dust lies in its sticky
and mucous nature (muous content) which holds the small par-
ticles together with an unusually strong power of coherence.
that -- to quote Cornet -- is a blessing for mankind, other-
wise infections would occur far more frequently. Sputum
which has dried on the ground and on various articles is
caused by careless spitting. This makes it necessary for
patients to be equipped with spittoons, which should also
be provided by stores, hotels, and other public places.

— Spitting into handkerchiefs should also be avoided whenever possible. These and other prophylactic measured for the prevention of the infection and spreading of tuberculosis were suggested by Cornet. He believed that most of the inhaled tubercular bacilli settled at the entrance of the respiratory tract due to their heaviness; also, passage through the nose and the vocal chords was very difficult. Only one bacillus out of a thousand manages to reach the depth of the respiratory tract and have a pathogenic effect in an infected spot only.

Extensive research on the importance of drop infection in the spreading of phthisis was done by C. Flugge (1, 2, 3, 4, 5) and his students (Sticher, Heymann, Latschenko, Neisser, Kirstein, Findel, Reichenbach, Ballin, and others) at the Breslau Institute of Hygiene. Whenever fluids containing germs are spattered, tiny drops penetrate the air. They are carried by minimal air currents, spreading the germs widely. A patient's speech, cough, and sneezing cause the formation of very fine drops which represent a type of air infection not considered heretofore. In his fundamental work on infection through the air, C. Flugge (2) wrote in 1897 that drop infection is far more important than dust infection, because germs -- even those most susceptable to dryness -- are communicative in their primary stages. The possibility of drop infection, however, excludes all diseases where the infection is localized outside the respiratory tract (gonorrhea, recurrens). Even the smallest drops containing germs may float in the air up to five hours. After this approximate period, they come in contact with some surface (walls, furniture, floors, the outside of the human body). The drop dries very rapidly on the surface, and it germs stick to it so firmly that an air current could not possibly remove them. Only through artificial means may they be reduced to dust and begin to mingle with the air again. According to Flugge, such cases are rare. In the case of drop infections, the most important factor is not the consumptive living space, but rather the excretion of the sick person passed into the air in the form of drops several hours previously. In spite of the extensive spreading of germs in the air, the chances of drop infection are very slight outdoors.

Two years later (1899), Flugge published the article, "The Spreading of Phthisis Through Dust-like Sputum and Through Drops Sprayed by Coughing," where -- influenced by the research of his student Sticher -- he placed more importance on dust infection. Nevertheless, he still considered fine drops containing bacilli more instrumental in causing tuberculosis in the human organism.

Before discussing dust and drop infection experiments in greater detail, we would like to refer to the publications of Hildebrandt, and particularly Buchner, who dealt with the penetration of various pathogenic micro-organism into the lung through an intact surface, and their multiplying within the organism.

Of the three main areas invaded by germs skin, intestine, lung Hildenbrandt (1888) tested the respiratory tract as the entrance. He sets out to answer four questions: 1. Do the micro-organism floating in the atmosphere reach the lungs undisturbed during respiration? 2. Can the micro-organisms penetrate from the alveoili into the intact lung tissue? 3. Can they multiply at this point? and 4. How do they move forward and produce a general infection?

To answer the first question, four healthy rabbits which had not had close contact with infection sources were killed. Individual portions of the respiratory tract were placed on suitable media in order to determine a possible growth of germs. While colonies developed in the oral and nasal mucous membrane, this was not the case in the lung tissue and trachea. Hildebrandt drew the conclusions that normally there are no microorganisms in the trachea and lung tissue, because the nasal cavity acts as a protective filter. To test the degree of protectiveness with a high bacteria content in the air, he had rabbits inhale pathogenic micro-organisms (aspergillus fumigatus).

For this experiment, he used a glass box with a cover which could be closed. A fan was placed on one side to whirl up the fungoid lawn in the glass container. The stir was increased with the aid of a goose feather used through the open top. Opposite the fan, that is, on the other side of the glass container, an opening served to connect the face of the animal by means of a concave cloth mask with the interior of the glass container. The period of inhalation lasted 35 minutes; the dissection took place from 30 minutes to four days after the inhalation. In this instance media containing trachea and lung tissue showed colonies of aspergillus fumigans. There were no colonies on media containing blood.

These experiments show that under normal circumstances the nasal cavity acts as a protector against the penetration of pathogenic micro-organisms into the depth of the respiratory tract. If the bacteria content in the air rises above normal, the protective function of the nasal cavity diminishes in direct relation to the increase of bacteria

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density in the air. To test the control of these results, Hildebrand had rabbits inhale pathogenic rabbit septicaemia bacilli and anthrax bacilli or their spores. These results were similar to the previous. An abnormally-high bacilli content in the air decreases the protective function of the nasal cavity, enabling bacteria to penetrate into the depth of the respiratory tract.

In testing the penetrating power of the micro-organisms from the alveoli into the lung tissue, Hildebrandt found that an intact lung surface does not provide a barrier. He found that the lung offers a favorable environment for the multiplying of only a few types of microorganisms; of the three types of microbes tested, only the rabbit septicaemia bacillus multiplied. In trying to answer the fourth question, he was able to show the importance of lymphs as passage to the bronchial glands (similarly as in the dust inhalation experiments); from there, penetration into the blood is possible. Nevertheless, he also considered the possibility of direct blood infection.

Buchner (1, 2, 3, 4), partially in a cooperative effort with Merkel and Enderlen, conducted extensive experiments on animals. Since their importance is fundamental, we shall relate them in detail.

Starting with the dust inhalation experiments by Arnold, and inspired by C. Flugge's (1) 1886 monograph where the thesis was established that bacteria cannot pass an intact lung surface, Buchner experimented with anthrax spores, chicken cholera stimulants, and septicaemia bacilli. He traced the route of inhaled bacteria in the lungs, and their passage into the blood. He was particularly interested in proving that the passage of bacteria through an intact lung surface and their entrance into the blood stream do exist, and not only in the case of those infection stimulants which, as the tubercle bacillus, experience a primary settlement in the alveolar wall.

In his experiments with pure cultures of tubercle bacilli, Robert Koch had found that these inhaled microbes penetrate into the respiratory tract as far as the alveoli. All his rabbits and guinea pigs which had been exposed to the inhalation of pulverized tubercles showed numerous tubercles in the lungs several weeks later. Macroscopically, these showed an alveolar growth at a specific stage of development. Animals which had been put to death or had died later had tubercles in the liver and in the spleen. There was no doubt that the entire organism had been infected

through the respiratory tract. Secondary localizations,
that is, tubercular metastases in other organs (spleen,
liver, kidneys, and so forth) could have been infected only
through bacteria in the blood stream. Since tubercular bac-
teria find the alveolar wall suitable for multiplying, that
is, a primary development of lung tubercles takes place
in animals tested, Buchner concluded that consecutive c
.. might possibly open abnormal communications, follow
by the passage of tubercle bacilli into the blood. Con..-
quently, in testing the penetration of bacteria through the
intact alveolar wall, only those should be used which do not
form flocks in the lungs.

In elaborating on the dust inhalation experiments
summarized by Arnold (cf. chapter on dust inhalation), Buch-
ner wanted to study the behaviour of inhaled micro-organisms
in the lung and in the organism. It seemed to him that the
purely mechanical process on the inhalation of small sus-
pended particles always remained the same, regardless whether
they came from coal, minerals, metal, tobacco, ground ultra-
marine, or dust containing bacteria and dried spores. For-
ces which move lifeless dust are also capable of conveying
animated bacteria dust, and passages which are open to the
one cannot remain closed to the other. In regard to inhala-
tion and passage through the lung surface into the bronchial
glands, lifeless dust should be compared to bacteria without
hesitation. From the purely mechanical point of view, a
bacteria cell is equally subject to pressures, tractive power,
and fluid streams as any other organic or unorganic dust.
The same applies to passage, and should there be a differen-
ce in this respect, then bacteria should be even more easily
conveyed due to their light weight. It should not be con-
cluded that in regard to their ease of movement and spread-
ing in the organism, bacteria cannot achieve more than life-
less dust. The movement capacity of coal particles and cin-
nabar granules represents only the minimum, a fact which ap-
plies to bacteria as well. Particularly in the case of
lymph glands, both clinical and experimental data show that
instead of stopping, infection stimulants, stream towards
the nearest glands and spread through the blood to the in-
ner organs. Neither should we disregard the important fac-
tor of the capacity to multiply on the part of pathogenic
germs which have penetrated into the lymph glands, or their
infectious effect. This may cause the otherwise adequate
filters to lose their dependability. Pathogenic germs which
do not multiply, however, are held back in the glands like
lifeless particles of dust, and are eventually destroyed.

There is no reason why inhaled bacteria which normal-
ly occur in the air should not reach the alveoli and be

passed into the lymph glands of the lungs without appearing in the blood or the other organs (lymphogenic removal). The common sacrophytic bacteria, like lifeless dust, are probably held back in the bronchial glands and do not (or barely) reach the blood. Consequently, these have an important prophylactic task.

A second possibility of penetration into the blood is a direct passage from the alveoli into the blood, that is, into the bordering capillaries. In the inhalation experiments (cf. chapter on dust) this possibility was either denied or seriously questioned. Thus tests had to be made to determine whether bacteria pass directly into the capillaries of the alveolar wall through intact surfaces.

Buchner experimented with the inhalation of dry pulverized anthrax spores and vaporized anthrax spores, anthrax bacilli, and chicken cholera bacilli. The microbes were first inhaled in the form of dust, then in the form of drops. We intend to discuss each of the two series of experiments separately. In the last paragraph, we summarize the results of all tests concerned with the passage of infection agents through intact lung surfaces.

The dry inhalation experiments dealing with anthrax spores presented some difficulty in method, since it was not easy to produce totally dry dust which at the same time was infectious. Finally the anthrax spores were dried to powder, which is effective in dusting (coal powder, lycoperdon) and served as agent in the pulverization process.

The dust was kept in calcium chloride to preserve complete dryness. The ratio between coal particles and anthrax spores was usually 1000:1, with lycoperdon dust 140:1. A small amount of the dust was injected subcutaneously to test its virus content.

Two kinds of apparatus were used in the inhalation experiments. The apparatus for mice had a volume of 3.3 liters; for guinea pigs the volume 13.6 liters. The latter is shown in diagram 1. The bottom of the glass apparatus is a tin-plate funnel (t). The spore dust is deposited by means of a glass tube (b), which passes through a rubber ring (g) located at the very bottom of the funnel. The animals are in a wire cage (c). The air is blown in by means of an ordinary fan. The air passes through a second vertical glass tube (a) to the lowest point in the apparatus, and stirs the dust deposit, diffusing it in the apparatus.

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Diagram I

Dust inhalation apparatus by Buchner and Merkel,
taken from Buchner and Merkel: Archiv fur Hy-
giene (Hygiene Archives), No 8, p 163, 1888.

The air exists through a plug of cotton wool which completely
fills the area between the glass neck of the apparatus and
the two glass tubes. Since the dust showed a great tendency
to settle everywhere within the apparatus, it was necessary
to shake and tilt it constantly during the experiment in
order to avoid a settling on the very bottom.

The quantities of dust used were very small, for it
appeared that large quantities conditioned foreign body
pneumonia and prevented the intended anthrax infection. The
pulverization and inhalation, the entire test, took only
ten to fifteen minutes every time. The quantity used in
one inhalation did not exceed .25g in the small apparatus
for mice, and .5g in the larger apparatus for guinea pigs.
The animals were exposed to the inhalation only once. Each
experiment was followed by a period of waiting until the

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dust particles floating inside the apparatus had settled.
Then the animals were cautiously removed and taken outside
to get rid of any dust particles which may have been cling-
ing to them. Each part of the apparatus was immediately
sterilized in a solution of mercuric chloride 1:1000.

The animals were put to death with chloroform 3 to
48 hours following the inhalation. They were washed with a
solution of alcohol and mercuric chloride and dried. The
lungs and the spleen were removed by means of sterile instru-
ments, and were placed in sterile containers. One section of
the lung was preserved in alcohol for a microscopic examin-
ation. Sterile scissors were used to cut the other section
into many (20 to 40) pieces, which were placed in a liquid
8% meat peptone gelatine, poured on a sterile glass plate,
and cultivated at 22°. The spleen was treated in a similar
manner. The advantage of this method is the possibility of
testing the bacteria content even if the microscopic examin-
ation should fail.

Inhalation experiments to test lung infection gain
validity only if corresponding controlled feeding experi-
ments prove that the infection was not caused through the
digestive tract by bacteria which had been swallowed. For
this reason, similar quantities of dust were fed to the
same number of animals of similar breed. Spore dust was
added to a mixture of bread and carrots. Chances of an in-
testinal infection were extraordinarily favorable as com-
pared to an air infection, since one group of animals inhaled
the maximum of 1/1000 of the pulverized spores, while the
other animals consumed the entire amount of spores given.
Since a remarkable majority of the latter stayed alive while
most inhalation animals died within a relatively short pe-
riod of time, it is impossible to assume an infection caused
by swallowed spores during the inhalation period.

The 13.6% pneumonia occurrences during the inhalation
experiments should be pointed out in this connection. In
his dust inhalation experiments (cf chapter on dust), Arnold
observed 19 cases (20.4%) of acute pneumonia in a total of
93 animals. Most cases (33%) were produced by sandstone
dust. However, we should bear the fact in mind that those
experiments were extremely long. Arnold assumes that the
origin of these pneumonia cases is the direct result of a
catarrh inflammation of the bronchial mucous membrane, that
is, broncho-pneumonia processes. In addition, the dust in-
halation may create conditions favorable not only to the
penetration, but also to the growth of specific infection

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By means of plate culture, Buchner has frequent-
... considerable amounts of bacteria (with the ex-
... anthrax bacteria) in inflamed lungs. The number
... bacteria detected in the lung tissue was partic-
... ... forms of pneumonia were present.
... was found. Pneumonia anti-
... retarding rather than favorable influence on
... of anthrax infection in the tissue of the
lung.

The atomization of liquids with a bacteria content
produces a very fine mist, parts of which transport the
clinging germs as far as the alveoli. This was evident in
the tests performed by Tappeiner and Robert Koch. There is
no need here for the cumbersome preparation of infectious
dust, such as the powerful drying process which can be sur-
vived only by true spores. The moist method, as exemplified
in this series of experiments, is just as suitable for an-
thrax bacilli as chicken cholora bacilli, or other types
of bacteria which perish easily through dryness. To as-
sure proper results, it was necessary to improve the custo-
mary method of atomization. It was particularly important
to avoid excessive moisture or saturation of the animal ex-
posed to the spray. Consequently, larger spray particles
were deflected, and only the minimal ones used. The appara-
tus used by Buchner is shown in diagram 2.
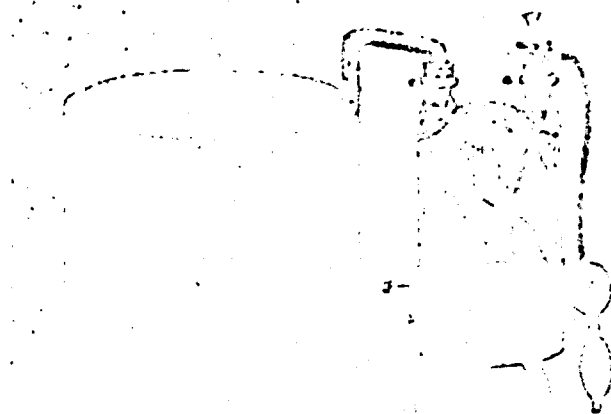
Diagram 2. Buchner's and ...len's atomizing
apparatus, reprinted from Buchner
and Enderlen: ...chiv f. Hygiene,
No 8. p 182, ...

The spray was produced by an ordinary atomizing apparatus by Bergson, that is, two upright glass tubes were placed on top of each other and attached to the bottom of a Wulff flask. The Wulff flask is cut off at the bottom and set up on a tin container (b). These two are connected to each other with a ringed shaped rubber band (g). Two rubber tubes pass through the neck of the Wulff flask (f). One of these (l) feeds compressed air to the atomizer; the other tube is equipped with a funnel to provide additional supply of atomizing fluid during the experiment. As soon as the spray is set in motion, an extremely fine mist penetrates the inhalation area (k) from the second neck of the Wulff flask and through the tube attached thereto. The mist is light enough to be borne upwards by the slightest air current. The tendency of the particles to deposit or settle is minute. Like cigar smoke, this mist may be conducted through a tube several meters long at moderate air speed. The animals were placed in a large (50-liter volume) tin kettle (k). A wire grating was placed several centimeters above the bottom of the kettle. The closure consisted of a large top cover with a glass panel to facilitate the observation of the animals. The top edge was padded with several layers of cotton wool to prevent the air supplied from escaping. Passing air is filtered by the padding. Following the inhalation, the animals appeared to be dry. They were placed immediately in an isolation cage. A mercuric acid solution was used to disinfect the entire apparatus immediately after completion of the inhalation.

Diagram 3. Spray apparatus by Buchner, reprinted from Friedberg and Reiter: Handbook of Pathogenic Microorganisms, second edition, p 502, 1912

Every animal inhaled only once. The duration ranged from 15 minutes to one hour. The inhalation consisted of 1) anthrax spores, 2) a type of hay bacilli, 3) typhoid bacilli, 4) cholera vibriones. Preliminary experiments were designed to determine whether the mist of a bacterioid liquid contained a sufficient amount of bacteria. Fresh plates of sterile gelatin media which had been exposed to the mist for a few minutes developed thousands of colonies two to three days later. Consequently, the mist transports bacteria, which means that atomizing does not destroy them. In order to determine the percentage of atomized liquid which reaches the test room, the liquid was directed into a glass container stuffed with hydrophilic gauze. The container was weighed before and after the experiment. The mean of the total amount atomized liquid was .5%; that is, when 100 cc were atomized by the spray apparatus, .5cc passed into the inhalation area in the form of spray.

These figures showed what small amounts of pathogenic germs are necessary to start an infection. Only in this manner was an insight gained into the amounts required in the feeding experiments. To avoid mistakes, the same types of bacteria were used as in the inhalation experiments. This accurate procedure along made it possible to prove from the intestines in rabbits and mice that even with chicken cholera, known to be highly infectious, the animals that died after inhaling were not infected by bacteria which they had swallowed. The inhalation of anthrax spores, for example, required a quantity of spores 300 times smaller to achieve the same effect in half the time.

Let us discuss the results of the two series of experiments. In every instance, a microscopic examination of the lungs 20 to 24 hours following inhalation (at the outstart of the infection process) showed that the inhaled microbes had penetrated into the lungs. The preparation received in the first series, however, definitely differed from the atomized ones. Dry pulverization had effected no infusion of anthrax bacteria in the capillaries, while the second series of experiments showed characteristic infusions. According to Buchner, there are two different stages. At first, the anthrax spores germinate. In the next phase, small groups collect under the alveolar epithelium. For the most part, they are infused in the blood capillaries.

The question arises as to how they passed into the blood stream. Three possibilities are plausible, yet the first one must be excluded. Nevertheless, we would like to mention it briefly. We could assume that the bacteria found

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in the lung capillaries did not stem from the spores that settled in the alveoli, but from those swallowed during inhalation and passing into the lungs from there. One point against this assumption is the time ($23\frac{1}{2}$ hours) between inhalation and the death of the animal. In the experiments where maximum quantities of spores were fed, none of the animals died until the fourth day. If this maximum amount needs such a long time to pass through the stomach and start an infection in the body from the intestine, then the minimal quantities which are swallowed during inhaling cannot achieve the same effect in half the time. Consequently, only two possible explanations remain for the origin of bacteria found in the capillaries. They were either transported by the lymphs to the bronchial glands and from there into the bloodstream, or else there may have been a direct passage from the alveolar wall into the blood. One possibility does not exclude the other; both could probably exist at the same time.

As already mentioned in the chapter on dust, the inhaled particles as a rule do not pass through the bronchial glands. Muskabluth, who made an intratracheal application of anthrax spores, found no anthrax bacteria in the bronchial glands 48 hours later. He concluded that they had moved on and had entered the blood stream. This conclusion is not necessarily correct, because the agents could have entered the blood directly from the alveolar wall. Buchner believes that the passage through the lymph glands is not an easy one, if at all possible, and totally dismisses the possibility of bacteria passing through the lymphs into the blood within several hours. The passage is relatively quick, however, when pathological changes in the lymphatic glands are present. This fact is in accordance with clinical data on the action of lymph glands in the human body.

Consequently, only one possibility remains: the direct passage of the bacteria into the blood. This assumption is probably the only correct one. If we consider the characteristics of the capillaries and the anthrax bacillus, we may assume that the growth takes place through the gaps which appear in the vascular structure under the influence of diseased agents.

The same gaps which provide a passage for erythrocites and leucocites during any inflammation also allow the infection agents, which are in the immediate vicinity of the vascular wall and exercise chemical irritation, to pass through. Since this penetration through the intact lung surface depends on activity of the bacteria, on their growth and multiplying, inhaled coal particles can never enter the blood

stream. A liquid stream or mechanical transport does not exist in this direction. Neither can apathogenic germs, even having reached the alveoli, ever pass through the capillary wall, simply because they cannot multiply in the tissue and fluid of the organism.

To avoid a misunderstanding, we would like to stress the fact once again that the assumption of a direct passage into the blood stream does not exclude the other possibility through the lymphs and bronchial glands. Both may exist simultaneously. Passage through the lymphs into the bronchial glands, which is a purely mechanical process, should be open to pathogenic germs as well as to lifeless coal particles and cinnabar granules. The same applies to apathogenic germs, as they are always present in the atomsphere. While lifeless dust particles and apathogenic germs are retained, reabsorbed, or perish in the bronchial glands, the fate of the pathogenic germs may be different. Thus a possibility of passage does exist for pathogenic germs.

The question remains which infection agents could penetrate the intact lung surface. Generally speaking, all blood parasites, that is, the infection agents which live in blood and have the capacity to multiply, should be able to do so. Primarily these are the anthrax bacteria, bacteria of mice and rabbit septicaemia, chicken cholera, recurrens, and malaria. Tubercles and mucous bacilli are not blood parasites, although tubercular bacteria were found in the blood of highly tubercular humans and highly infected animals. Consequently we cannot assume a direct passage of inhaled tubercles into the blood stream. This has been confirmed by inhalation experiments. A general infection does not develop immediately in the animal; it is initially preceded by localized tuberculosis in the lung. Localizations in the inner organs are secondary. It is highly probable that these were not caused by direct passage into the blood, but rather through the lymphs, accompanied by successive tubercular infection of intrapulmonary lymph follicles and bronchial glands. From here, the fully mature bacilli pass into the blood of the animal species, provided the appropriate receptivity exists.

In this connection, Buchner experimented with the inhalation of pulverized mucous bacilli.

An agar culture was suspended in 40 cc water and inhaled by two guinea pigs for half an hour. Six days, later, both animals reacted with audibly heavy breathing and a

brownish discharge from the nostrils. 14 to 39 days later
-- heavy loss of weight and death.

The dissection showed more remarkable findings in
the first animal. A singular circumscribed mucous nodule
was found in the upper right section, while the remainder
of the lung totally intact. The lung of the second ani-
mal was intact, and yet signs of early miliary mu-
cous eruptions were present in the spleen. There is only one
explanation for the mode of infection in this case; namely,
the bacilli forced their way into the blood stream through
the lymphs. There was no primary localization in the lung
tissue, as is usually the case with tuberculosis. Buchner
also experimented with the action of inhaled pus cocci,
typhoid bacilli, and cholera vibriones in the lung, and
their passage through the lymphs and the blood. Since no
exact results were observed we shall omit them at this point.

In this connection, we would like to discuss the re-
sults of experiments with intratracheal injections of Fried-
lander bacilli. These were carried out in 1905 by J. Kowaki
at the Marchand Institute in Leipzig. The results may be
summarized as follows:

1. The lung may be infected through a direct injec-
tion of bacilli into parenchyma, or from the respiratory
tract. In most cases, bacilli pneumonia in the human is
probably an aerogenous infection. A haematogenous infec-
tion through these bacilli, however, should not be dismis-
sed entirely, since they may easily reach the blood stream
from any primary disease center.

2. Capsular bacilli succeed in causing inflammation
of the lungs only at high maturity or in large quantities,
otherwise additional predisposed instances in the lung tis-
sue (trauma, cold) are necessary. These instances are pro-
bably of great importance in bacilli pneumonia in humans,
since the bacilli do not reach the lung in as great quanti-
ties as they do in animals.

3. Bacilli which reach the lung are accepted by the
lymphatic system through the alveolar epithelium without
difficulty. This happens more quickly than the absorbtion
of coal pigment. Having reached great virulence, a portion
of the bacilli is once again eliminated into the alveoli, in
order to inflame the parenchyma (serous exudation, leucocite
accumulation). Another portion of the bacilli degenerates
in the lymphatic system and looses its virulence. This is
particularly the case in an intact lung tissue. Other bac-
teria reach the blood stream and cause a general infection.

4. Following intratracheal injections of guinea pigs, only flecks of pneumonia were to be found. This may be traced back to two possible reasons. Either the bacilli perish rapidly when the lung tissue is intact, that is, before extended pneumonia occurs, or the animals succumb to a general infection before the pneumonia process spreads over an entire lobe. A passage of bacilli through the bronchial wall has never been proven. This shows how difficult it is to use pathological-anatomical findings for the analysis of initial inflammatory processes following the penetration of bacteria into the lungs. For this reason, we shall return to the hygienists who conducted numerous experiments to study the behaviour of the tubercular bacillus outside the lung and following inhalation into the lung of guinea pigs.

Neisser had shown that extremely fine dust mixed with phthisic sputum when influenced by weak air currents can carry tubercle bacilli in a live, virulent state. It was necessary to find out whether germs contained in tubercular sputum can be carried by moderate air currents in an infectious state and produce inhalation tuberculosis in animals. Sticher (1899), a student of Flugge, carried out two series of experiments in this connection. Since earlier experiments had usually shown negative results, he used a strong air current in the first series and a weak current (which corresponds more to natural conditions) in the second series to transport dust containing bacteria.

The apparatus used in the inhalation experiments with strong air currents is reproduced in diagram 4.

An ordinary round rubber bag (A) with an opening was used. A second round opening was made in the bottom of the bag, directly opposite the original opening. By means of vulcanizing, a collar-like attachment (B) was placed around this hole in a position which corresponded to that of the wooden ring. This flexible ring, which could also be closed tightly, was designed for the neck of the animal. The wooden ring in the bag had a rubber stopper equipped with two glass tubes. The air from a dust developer was forced through one tube, and used the other tube to escape, having passed the extension (C) and a glowing copper spiral (D). In the first three experiments, the dust developer consisted of a flask (E) which was constantly shaken to raise bacillic dust. During the next eight experiments an ordinary ice bag was used. It contained torn canvas rags with dry sputum. Air was forced in through an attached tube. Carrying dust particles, the air forced its way into the inhalation chamber

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cannot tell above. In the first three experiments, where it
was difficult to produce an exact distribution of damp sputum in dry dust and to avoid the formation of rough particles,
an extension (G) was plugged in between the dust developer
and the inhalation chamber. It was designed for the settlement of the rough particles. In all experiments, the tube
leading into the inhalation chamber had a rectangular bend
in order to avoid a direct entrance of the dust-bearing
current into the respiratory tract of the animal. The fan
was compressed rhythmically, approximately 32 strokes per
minute, at regular intervals. In order to obtain an idea
of the approximate air current speed, we determined the amount of water forced out by one stroke of the fan (50 cc).
The speed of the air current in the 4mm wide tube leading
into the inhalation chamber was approximately 1m at the time
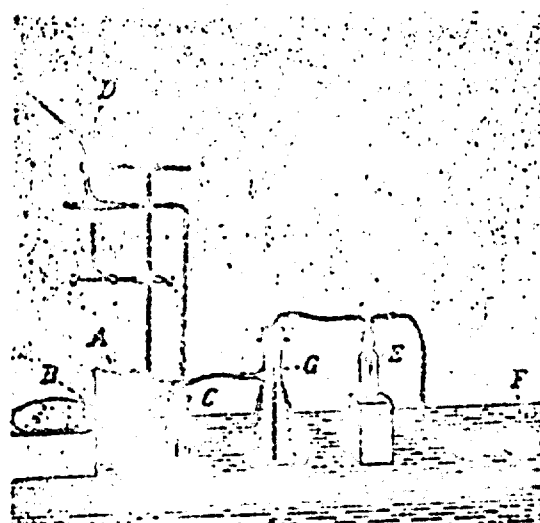of the stroke.

Diagram 4. Sticher's experimental apparatus,
reprinted from Sticher: Zeitschrift fur Hygiene (Hygiene Periodical), No 30, p 166, 1899.

The animals used were guinea pigs. The duration of
the experiments was upt to 60 minutes. Tubercular sputum
was dried on canvas rags until no trace of moisture could
be seen or felt. The dry sputum particles were then loosened mechanically and fed into the dust developing apparatus.
Unless natural death followed within several weeks, the
animals were put to death at different intervals.

In every case, the findings showed lung and bronchial tuberculosis, sometimes tubercular flocks in the extrapulmonary organs. This proved that tubercular bacteria settled on dust can reach the lungs in an infectious state when carried by strong air currents. The negative results of earlier scientists were caused by the dust boxes used. They had been too large for the animals, providing too weak a concentration of dust. Due to their small breathing volume, the animals had had little chance to inhale dust particles laden with bacteria.

The second series of experiments used weak air currents in order to imitate natural conditions as they exist in aerogenous infections.

Sputum was dried not only on canvas rags, but also on pieces of wood. The latter was to reproduce conditions which exist when sputum dust is raised on the floor. The distance between the pulverizer and the inhalation chamber was extended, because the greater the distance, the fewer are the chances of infection, since the larger particles have a tendency to settle and the bacteria run the danger of being damaged. The most important modification, however, was the change in the speed of the air current. The intention was to reproduce the speeds prevailing in ordinary living space. The sputum was dried to a maximum, that is, the sputum bacilli were dried in a calcium chloride exicator until no more loss in weight could be determined. The drier the dusting matter, the better the chances of its being inhaled.

The apparatus shown in diagram 5 was used in these experiments.

The apparatus consisted of the following 6 parts: pulverizing chamber, dust conductor, inhalation chamber, condensor, disinfection and aspiration arrangement. As in the previous experiments, an ice bag (A) served as a pulverizing chamber, a tin tube (B), 2cm in diameter and 1m in length, was the dust conductor; it connected the pulverizing chamber with the inhalation chamber (C). The inhalation chamber consisted of an oblong tin box with 4 rectangular and 2 square surfaces which remained open. The front opening could be closed by two insertions, each of which contained a semi-circular aperture. The guinea pigs which were placed into the box through the back opening could be held by inserting their heads in the front attachments. A small tin cap closed the back opening. A larger

Best Available Copy

... ... placed over the front part of the box, creating ... ... for the heads of the animals. The inhalation chamber was connected through the condensor (D) an Erlymeyer piston, the bottom of which was covered with sterile water, and the disinfection arrangement (E), a flask filled with sulphuric acid, with the aspiration chamber (F). Aspiration ... ... produced by means of a tin cylinder (F) containing ... ... and equipped with a drain.
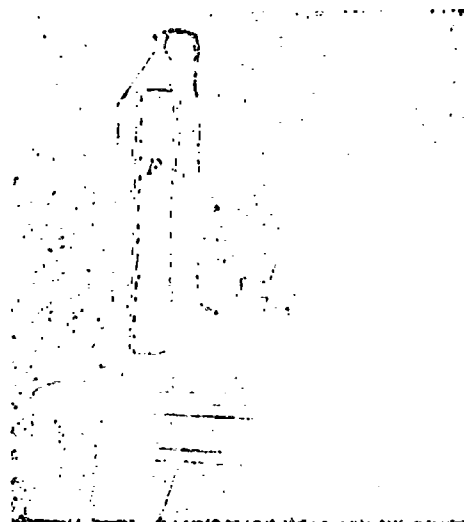

Diagram 5.   Sticher's apparatus, reproduced
             from Sticher: Zschr. f. Hygiene,
             No 30, p 178, 1899.

The experiments were carried out in the manner outlined below. After the dried pieces of wood and rags had been placed in the dusting bag (which was dry, as were all parts of the apparatus except for the condensor), the guinea pig -- with the neck hair shorn off -- was brought into the inhalation chamber. Suction was produced by draining the water from the cylinder. Manually (through rubbing or shaking) the dust carriers (pieces of wood or rags) were freed from the bacterioid dust and whirled up. The presence of fibers in the tube leading from the pulverizer showed that suction of dust was actually taking place.

The velocity of air was 10cm per second. Occasionally stronger currents were used to reproduce conditions similar to draughts in a room (opening a window).

As a result of the slight bacteria action, animals used in this series of experiments did not die spontaneously. Several months after the completion of tests, a dissection revealed tuberculosis of the lungs, bronchial glands, and the rest of the organs only in a few cases. On the other hand, animals injected with the dust from the condenser showed a tuberculosis infection in every case. Sticker concluded that the driest possible tubercular dust, carried by weakest air currents, may cause an infection; that is, even the finest and lightest dust particles which float in the air for a long time may carry virulent tubercular bacilli. The small number of inhalation infections in the second series is due to the fact that the dust concentration was weaker as a result of a lesser whirling effect and a greater distance between the pulverizer and the inhalation chamber. Since the animals spent only one hour in the inhalation chamber, the chance of becoming infected through the inhalation of tubercular dust suspended in the air was minimal.

Nenninger, another student of Flugge, studied the penetration of bacteria into the lung through the inhalation of drops and dust, and thought it necessary to clarify several points before starting the actual experiments. Previously, the lungs of health animals and humans had generally been considered free to germs, which contradicts the fact that the surface of the lungs is constantly in contact with more or less germ-containing outside air. Hildebrant and Klipstein were of the opinion even before Nenninger that pathogenic germs are often to be found on the inner surface of the lungs, yet these were eliminated in no time. Barthel maintained, however, that the lungs of healthy humans should generally be considered as being free of germs, although the large and medium bronchi always contained pathogenic germs. Nenninger examined the term content in the lungs of three pigs, three cows, and one rabbit, and generally found a low germ content.

Experiments with the inhalation of drops were carried out as outlined below.

Prodigiosus germs were suspended in a Buchner spray apparatus containing a 6% solution of NaCl and atomized for a period of 10 minutes. The animals -- guinea pigs or rabbits -- sit in a tin box, equipped with a round opening for the neck of the animal. The atomizer opening is 40cm opposite the head of the animal. Immediately following the inhalation, the animals are put to death through a stroke on the neck (medulla oblongata), the spray infected head is

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wrapped in a cloth steeped in a solution of mercuric chloride, and the animal is removed from the box. The skin is sterilized by placing the animal in a container of mercuric chloride solution. Under sterile precautions the lung, the main bronchus, and trachea were cut into segments. Every part was placed in a tube containing 1cc bouillon, there it was mashed using a strong platinum needle, and poured on an agar plate.

The experiments showed that the bacteria drops in the inhalation stream reach even the finest branches of the respiratory tract without any difficulty. This had already been determined by Buchner and Enderlen, although they did not test the germ content in immediate cultures. Neither did Nenninger succeed in determining accurate numbers in the germ content of the individual portions of the respiratory tract, which was partially due to the experimental technology.

In subsequent inhalation experiments, the attempt was made to determine the actual quantity of germs brought into the respiratory tract.

The prodigiosus germ used in the drop inhalation experiments was unsuitable, since it cannot stand up to the high degree of dryness necessary for pulverization. For this reason, the spores of a different type of bacteria (magatherium) were used, in Kabierske's pulverizer. A compression five seconds apart sufficed to keep the head of the animal in a constant cloud of smoke. About ten minutes after the completion of inhalation, the animals were put to death and skinned. Dissection took place in the drop inhalation series.

In this case, too, Nenninger found that the bacterioid dust had forced its way into the deepest portions of the respiratory tract. The number of colonies, however, was considerably smaller than that in the drop inhalation series, although in both cases the agar plates appear to have a similarly dense growth.

Finally Nenninger conducted aspiration experiments, in which he brought bacterioid liquid into the mouth of an animals by means of a cotton wool swab. He plugged both nostrils of the rabbit with cotton wool and wax. The trachea was pressed back several times until the animal was gasping and in doing so inhaled the bacterioid liquid from his mouth. The experiment was completed in five minutes, and the animal was put to death. The result surpassed all expectations:

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from the top to the very finest intricacies of the respiratory tract a large number of germs was found. This rendered the proof that forced respiratory air which brushed through the mouth cavity could separate drops containing germs and carry them into the depth of the respiratory tract.

The human pharynx particularly offers the opportunity for the formation of drops. Secretion follows the respiratory stream, then exspiration effects a dusting of the drops to the outside, inhalation to the inside. To be more specific, the inhalation stream, provided it finds sufficiently thin secretion, easily separates the drops and carries them into the depth of the respiratory tract. The drops, however, should not be too large, otherwise protective reflexes such as coughing, sneezing, and so forth, set in. The content of pathogenic germs in the mouth cavity points to an important source of lung infections. As Birch-Hirschfeld stressed, the mere presence of germs in the lungs does not start an infection. A local disposition is necessary. This applies particularly to certain bronchi at the top of the lung. Their unfavorable position in relation to the main bronchus and the trachea makes them particularly prone to the settlement of secretions and of foreign matter from the outside, such as infectious dust particles. The bronchial mucous membrane must be damaged or its resistance impaired before a tubercular infection may develop.

Similar experiments using Barthel's apparatus were conducted by Hartel and Herrmann at the pathological-anatomical institute in Vienna (Prof. Weichselbaum). These scientists did not subscribe to the idea of aspiration of liquid particles as found in the mouth and pharynx, and believed that the germs found in the lungs stemmed only from the drops suspended in the atmosphere.

Following the lecture held by E. von Behring at the convention of German natural scientists and physicians in Kassel in 1903, and the research done by Calmette in France, unsurpassed importance was ascribed to feeding tuberculosis. The widespread opinion arose that phthisis is caused not by the inhalation of tubercular germs, but rather through either a direct intake from the mouth and pharynx, or, following direct swallowing, through the intestine. Von Behring maintained that tuberculosis originated through the intake of nourishment containing tubercles into the intestine itself, or if no infection erupted here, through the passage of agents from the stomach lymph glands into the bronchial glands and the lung. Consequently, it appeared necessary to undertake comparative study of feeding and inhalation

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-tuberculosis. Findel and Kuss rendered valuable contribu-
tion in this respect.

H. Beitzke took a critical stand to the view of a
direct tubercular infection from the mouth and pharynx into
the lungs. Starting with statements of various authors
who maintained that aerogenous infection played a minimal
role, he tried to discover whether an infection path did
indeed exist from the mouth and pharynx over the lymph glands
of the neck to those of the thorax, that is, the lymphs of
the lungs and the bronchial glands. Having observed and
experimented with dissections of children, he concluded that
no lymphatic paths led from the cervical lymphatic gland
chain to the bronchial glands, and that a tubercular infec-
tion of the lungs coming from the lymph glands in the neck
could pass only through the upper trunci lymphatici, venous
angles, cava superior, and arteria pulmonalis. The infec-
tion of the lungs or bronchial glands in a child was caused
by the intake of liquid particles containing germs. The
origin of these liquid particles should be traced back to
the intake of tubercular foods or infections caused by dirt.

Findel, who was working in Flugge's institute in Bres-
lau, first had trachotomized calves and dogs inhale tuber-
cular drops, intending to prove that the infection had in-
deed originated directly through inhalation, rather than
through the secondary ways cited above.

For this purpose, a thick rubber tube was pushed as
far as possible into the inner tracheal channel of a trach-
eotomized calf. A large tin funnel was set on the outer
end of the tube. A Buchner spray apparatus was placed at
equal height in front of the funnel. Its outlet tube was
connected through a rubber tube to a glass tube which led
into the funnel, enabling the fine mist penetrate along with
the inhalation stream. Experiments on tracheotomized dogs
were carried out in a similar manner. For the sake of com-
parison, the animals were fed specific quantities of nour-
ishment containing tubercles.

Even these preliminary tests showed that the inhala-
tion required considerably less than .141mg = 4,900,000
bacilli to produce tuberculosis in the entire lung four weeks
later. On the other hand, the feeding of 172mg = 6020 mil-
lion bacteria had not effect whatsoever.

In the actual experiments without tracheotomy, that
is, without a sharp separation of the two infection paths,
the inhalation infection showed similar positive results.

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The apparatus for the inhalation was constructed with the help of Prof. Reichenbach, and is reproduced in diagram 6. The arrangement is based on the principle that the animals inhale from an air current which increases with constant velocity. The bacteria concentration remains constant throughout the experiment.
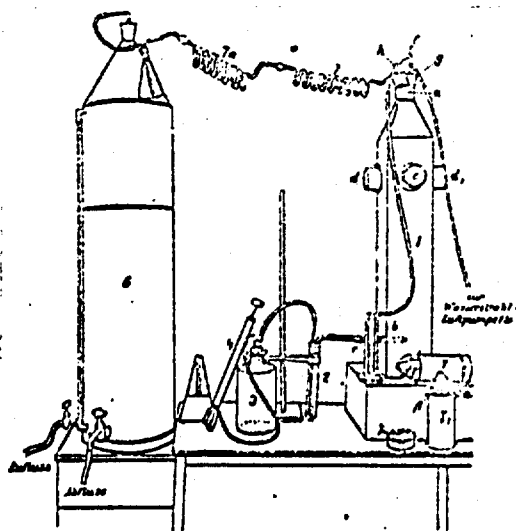

Diagram 6. Findel's apparatus, reprinted from Findel, H., Ztschr. f. Hygiene, No 57, p 125, 1907

The pyramid-shaped inhalation tower (1) has a round attachment (a) at the top with a thrice-bored rubber stopper. From here, a tube (g) leads to a strong water-jet pump, a second tube (h) to a manometer (c), and a third tube (f) to a large aspirator (6) which can draw any desired quantities of air from the tower. The bacterioid mist is produced in a Buchner type spray (2). It is set in operation by a bicycle pump with the aid of a 3-liter volume flask serving as a wind chamber. Close to the bottom, the spray passes into the tower (b). The air containing bacteria rises slowly in the tower and exits at the tops through the air pump. The pump is set to keep a constant pressure of 20mm water jet in the tower. If the presure is maintained constant and the quantity of air fed through the spray the same in the unit of time, then the velocity of the air current and with it the bacteria content in the unit of volume also remain constant.

The guinea pigs are placed in a cylinder (y) to make sure that they are allowed to breathe for an accurately

measured period of time. A rubberized air-tight cover fits over the back end of the cylinder. The front part has a basket-like wire arrangement (a) to hold the animals inside. The upper third of the tower has two openings (d, d'), each of which may be covered with an air-tight cylinder to allow the guinea pig's head to protrude into the cylinder by several centimeters. Glass panels (e) are installed at that height to allow observation of the animals during the experiment. There is no danger in inserting or removing animal containers or of loopholes in air-tightness as long as a negative pressure prevails in the apparatus. For this reason the negative pressure had to be controlled and maintained constant.

Since the spray used very little liquid, the apparatus was kept in operation for hours without difficulty, allowing great variations in the number of bacteria inhaled. The animals in the apparatus were totally relaxed and breathed under completely normal circumstances. There was absolutely no trace of "forcing" bacillic mist into the lungs.

In order to find the number of tubercular bacilli inhaled by a guinea pig, the germ content in the deposit of the Buchner spray and in the lungs of a guinea pig, or that in the Williams tube (7, 7a) was determined. The findings showed that per one liter air the two William tubes caught one 12,000th of the germs in one cubic centimeter of the inhalation. On the other hand, one 14,000th of this germ quantity passes into the lungs of a guinea pig. Further study showed that the upper part of the tower contained only 25% of the germs which had passed in through the bottom. Consequently, 75% are lost during the rise in the tower.

The method outlined below was used to produce the inhalation solution containing tubercles. One hundred MG of a four-week-old tubercle culture were mashed most thoroughly, first without water, then with a drop-by-drop addition of sterile distilled water. This was done in agate mortar and increased to 20cc. Thus a cubic centimeter of liquid contained 5mg of bacteria culture, that is, 175 million bacilli. Findel determined the number of bacilli following numerous counts in one weight unit. The result was as follows: one milligram of a tubercle culture raised on bouillon, which had been freed from superfluous fluid through pressure applied with a platinum spatula, contains 35 million bacilli.

The deposit mentioned above was successively thinned in subsequent experiments. The smallest dosage used still

contained .125mg=4,375,000 bacilli per one cc of atomizing liquid, that is, 313 bacilli per one liter air (if the apparatus removed .11 cc liquid in 10 minutes during the passage of 140 liters air during that period). Finer gradations in the number of bacteria inhaled were achieved through various measurements of the time of exposure. In one of the last experiments, one cc contained .025mg atomizing fluid, that is, 875,000 bacilli. Thus, one liter of air contained 63 bacilli.

Bacilli designed for feeding were removed from the William tubes. The expelled laevulous solution was mixed into a gruel with carrots and given to the animals after they had been kept hungry for 20 hours. In most cases the food was eaten up. The dosage surpassed that in inhalation by far; it contained between 19,000 and 322,000 bacilli. Five of the 14 animals received the entire dosage at one feeding; it was given to the nine remaining guinea pigs on five successive days.

Eighty-three adult guinea pigs were used in the inhalation experiments. They inhaled dosages ranging from 20 to 280,000 bacilli. All animals that had received over 62 bacilli and did not die during the first 28 days of an intercurrent disease, showed a heavy, macroscopically visible tuberculosis. After 28 days, tuberculosis had localized almost exclusively in the lungs and the bronchial glands, while only the spleen contained solitary tubercles. After 50 days, the tuberculosis had spread through all the organs. Of the six animals that had received smaller dosages (three animals 40, and three animals 20 bacilli each), only two showed an infection and macroscopically visible changes. The definitely lethal dosage for adult guinea pigs contained 62 bacilli; smaller dosages up to 20 bacilli promised no definite results. Findel was of the opinion that one bacillus sufficed to infect very young guinea pigs.

Dosages of 19,000 to 382,000 bacilli were used in the feedings. None of the fourteen animals showed changes that could be described as being tubercular, although they were observed up to 174 days. The lethal inhalation dosage was 1/6000 of the ineffective feeding dosage, and that which led to an infection amounted to 1/19,000.

Kuss, who also conducted comparative inhalation and feeding experiments with tubercular bacilli, used the apparatus shown in diagram 7.
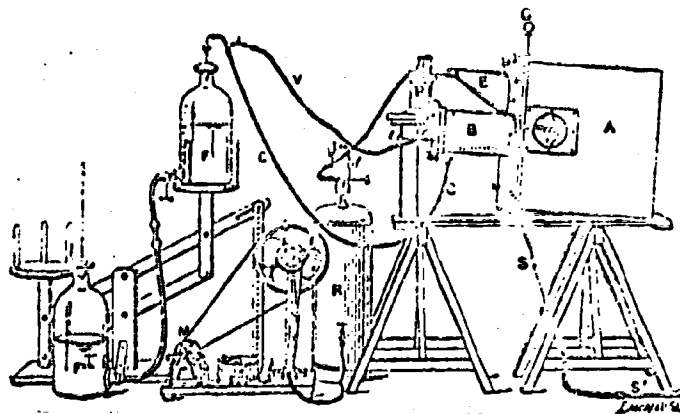
Diagram 7.   Apparatus by Kuss, reprinted from
             Kuss: VI. Internationale Tuberku-
             losekonferenz (Sixth International
             Tuberculosis Conference), Vienna,
             19-21 September 1907. R. Mosse-
             Verlag, Berlin, p 80, 1907.

A duct tube (E) is attached to a large tin box (A)
with a volume of 152 liters and an observation window (m).
A container (B) is attached to accommodate the animals.
The atomizer (P), which operated at three atmospheres abso-
lute pressure, sprayed 4 cc at an air passage of five liters
per minute. The air exits from the inhalation box through
tube connection (s, s'). Two guinea pigs sit side by side
in the containers (B), with only their heads reaching the
inhalation box. At the time of exposure, closure (G) is
raised and the back wall (t) pushed to the front, enabling
the head of the animal to reach the box (A). The guinea
pigs inhale the bacillic mist under normal circumstances.
During the entire inhalation process, the aspiratory (F,F')
sucks the air from the inhalation box (A) through a tube
(C-C') which starts between the heads of the guinea pigs.
A portion of this tube is of glass and stays in the animal
container. It is filled with carrot pulp (9g to the length
of 12cm). This filter held back all particles of air which
had been sucked in. In addition, the feeding of the infect-
ed carrot substance provided the opportunity to test the
virulence of the bacteria which had travelled that distance,
and to compare the dosage required for an oral infection.

     The experiments on guinea pigs were first conducted
with soot and Indian ink, then with tubercle culture deposits.

Two conditions are necessary before tubercular re-
search done on animals may be applied to tubercular contag-
ion in humans, namely: 1) the infected drops should
be inhaled for only a short period of time without exposing
the animals to the direct atomizer jet; 2) during the test-
ing period, at least the same quantity of bacilli should
be led into the inhalation chamber, filtered, and fed to
the animal. Kuss discovered that the inhalation of
virulent bacilli always caused tuberculosis
of the lung. Under similar conditions, inhalation is infi-
nitely more effective than oral intake or even subcutaneous
injection. Tuberculosis caused through inhalation comes
from the air. Bacilli which settle on the pharynx during
inhalation and are then swallowed play a secondary role as
disease agents.

Following Findel's research of the inhalation of tu-
bercles in liquid drops, Kohlisch (also a member of Flugge's
institute) experimented with the inhalation of dry tubercu-
lar dust (1905). His primary goal was to clarify the effec-
tiveness of dust infection as compared to drop infection.
Furthermore, he wished to determine the percentage of tuber-
cular dust that is held back at the entrance to the respira-
tory tract and the larger bronchi, and the percentage that
actually reaches the finest bronchi, and the number of tuber-
cular dust particles necessary to produce tuberculosis.

Initially Kohlisch used a sprayer which is reproduced
in diagram S.

Glass funnels are attached to the top and bottom of
a tin cylinder (A). The guinea pig sits in a box (B) which
closes at the front through a wire screen. The dust is
driven from the U-tube (C) to the apparatus by fan (D). A
movable tube (E) makes it possible to raise the dust, which
settles on all surfaces and in all angles. A hose (F) con-
nects the apparatus with a 70-liter volume tower containing
water. This water is drained at a speed necessary to draw
the air containing dust from the apparatus (A) through ster-
ile cotton wool filters (G). The end of the suction tube
is close to the nose of the animal. At the top of the upper
glass funnel, there is another cylinder (H) containing cot-
ton wool to catch the bacillic dust as the air escapes.

Kohlisch used dust from a cotton mill. This dust con-
sisted mainly of pulverized particles from seeds, cotton fi-
bers, and a number of fine elements that could not be ac-
curately defined. It was impregnated to the point where 1 mg
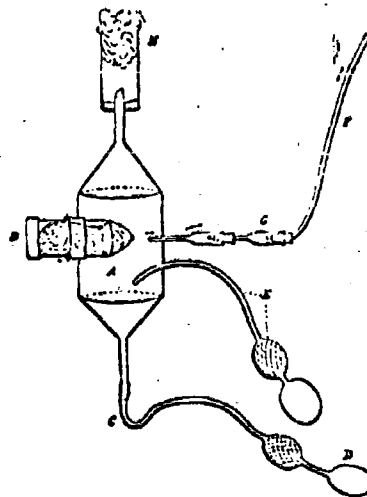dust contained 120 million spores. Every experiment lasted

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Diagram 8. Spraying apparatus used by Kohlisch in the first series of experiments, reprinted from Kohlisch: Ztschr. f. Hygiene, No 60, p 513, 1908

ten minutes, and with the aid of both fans a thick cloud of dust was maintained in the apparatus throughout. The animal was put to death immediately after completion of the experiment. The lung was removed, weighed, mashed in mortar, flooded with bouillon, and poured out on gelatine plates. A similar procedure was applied to filters and cotton wool stoppers. After sufficient growth had occurred on the plates (approximately 48 hours), they were counted, and the ratio between germs in the lung and in the filter was determined.

Results showed that on the average 7% of the germs contained in one liter of air reached the lungs. This was definitely less than in experiments with sprayed drops, where on the average 35% reached the lungs (Findel). The minimum effective dosage was also considerably higher in dust infection than in drop infection. While the latter required approximately 60 bacilli to start the infection, 260 bacilli were necessary for a dust infection.

It became apparent during the experiment that the apparatus needed improving in some respects. The cloud of dust, for example, did not last long enough in the apparatus. When smaller quantities were used, it was driven after half

a minute by the escaping air into the cotton wool in cylin-
der (H). A change of suction filters showed that during the
eighth and ninth minute the number of germs to be filtered
had decreased to less than 1/10 of those during the first
minute. With Prof. Reichenbach's help, Kohlisch improved
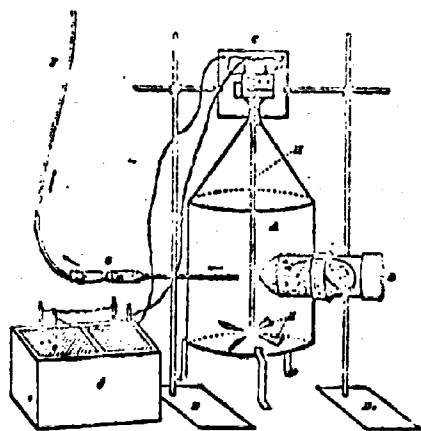the sprayer by constructing the apparatus shown in diagram 9.

Diagram 9. Improved spraying apparatus by Koh-
lisch, reprinted from Kohlisch:
Ztschrf. f. Hygiene, No 60, p 518,
1908

(A) is again the breathing chamber; however, it
has only one funnel at the top. At the bottom, it may be
closed horizontally. It is screwed to the table. (C) is an
electric motor driven by an accumulator (J). The extremely
long axis of the motor passes through the funnel and close
to the bottom of the box. A wind wheel (E) is attached to
this end. This turns clockwise, raising the dust from bot-
tom to top. The motor is supported by stands (D). The hose
(F) passes again to the water tower. Through it, air is
sucked from the apparatus through filters (G).

Initially this apparatus failed to improve the re-
sults. This was ascribed to the dust used. In respect to
suspendability, the cotton dust was composed of different
elements. The quantities of dust suspended varied constant-
ly, causing differences in the matter inhaled. In order to
imitate natural conditions, a mixture of apartment dust and
fine cloth fibers, obtained by pulverizing a handkerchief,
was used for inhalation in the following series of experiments.

The results once again showed the superiority of drop infection.

While tubercle bacilli which reached the lungs in drop form almost without exception caused tuberculosis, only a relatively small fraction of dust bacilli achieved the same effect. The difference was explained by the fact that dust particles, which settle on the wall of the bronchi, act as an irritant to set off vibrations which in turn move them before the bacilli have a chance to free themselves from the mucous and are capable of causing pathogenic changes. Also, the process of drying and pulverizing may have caused a partial weakening of the tubercular bacilli. The liquid drop of mucus containing bacilli, however, immediately mingles with the bronchial mucus, which is of the same consistency. For the same reason, the drop does not set off vibration due to irritation, and the tubercle bacillus can immediately -- undiluted and fully virulent -- attack the unsuspecting epithelium. Dust particles are more likely to be driven upwards and swallowed. In addition, due to their relatively large diameter, dust particles often remain in the nasal and pharyngeal region, making the infection of lymph glands in the neck possible. The swallowed portion of these tubercles seldom suffices for an intestinal infection.

In later years, B. Lange revived the entire problem of aerogenic infection of tuberculosis. He experimented on animals and test tubes for this purpose. There are three publications dating back to 1925 which we intend to discuss in some detail. Together with K. H. Keschischian (1) Lange in his first publication thoroughly treats the importance of drop infection. He is particularly interested in what quantity finest drops and dust are capable of penetrating into the lungs after having been inhaled.

With test tubes he studied the ability of drops and dust containing germs to pass through angular tubes of various widths.

The apparatus was similar to that of Findel and Kohlisch. A fine spray of bacteria or suspendable dust of a bacteria and talcum mixture was passed through a bent glass tube under moderate pressure in the lower portion of a tin tower with a square bottom surface. The spray apparatus (one similar to the Buchner spray and the other a glass flask partly filled with dust) were situated -- as the inhalation tower -- in an air-tight glass box.

Weak suction (1 liter per minute) of a water-jet pump
drew the air from the top end of the tower. It passed
through 10-20 cm long spiral glass tubes, 10 or 6 mm in
diameter. An Erlmeyer flask was placed between the glass
tubes and the water-jet pump. A solution of NaCl in the
flask and cotton wool filters in the drains served the pur-
pose of withholding all germs. The drops were to be caught
by a holder on the bottom of the flask in order to determine
their size and density.

Every stroke of the airpump brought approximately
1 cc to be atomized. In addition to the smallest drops of
2-20u, there were large amounts of drops between 20 and
100u, as well as 100 and 1000 u. There was no difference
between the related media in relation to the size of the
drops formed; only the absolute size of the smallest drops
of 2-20u, there were large amounts of drops between 20 and
100u,. as well as 100 and 1000u. There was no difference
between the related media in relation to the size of the
drops formed; only the absolute size of the smallest drops
was smaller with the application of fluid containingalbumen
and sputum than with watery eosine solutions.

Since the consistency of sputum is unsuitable
herewith. 1) it was determined up to what size the drops
may be in order to be taken by air currents from their
course and driven to a specific cavity. 2) Was it possi-
ble to determine the size that enables them to overcome
strong hindrances? 3) Beyond what size could they no long-
er overcome light hindrances? -- Even though these experi-
mental conditions cannot be applied to humans in their en-
tirety, we do obtain an idea of the quantity of cough drops
inhaled by a human, and of the extent of penetration that
should be taken into consideration.

Preliminary tests of the floating capacity of spray
drops supported Chausse's findings. After several seconds,
all drops over 200 u fell to the group; after several min-
utes, only drops up to 20 u, and after hours, only drops
of very few u in diameter had remained in the air. The
smallest drops could still be seen in the air one to two
hours later. The bulk of the cough drops, however, ranges
in size from, 100 to 500 m. Even the smallest bronchial
drops are supposedly never smaller than 10 u. The floating
duration of drops coming from a Buchner spray was estimated
at several hours; bronchial drops, however, remain sus-
pended in the air only several minutes. Their capacity to
fly is also limited. A holder placed at a meter's distance
from the coughing person, barely manages to catch any drops.

The flying capacity of spray drops, on the other hand, is unlimited. According to Flugge, air currents of only a few milimeters per second suffice to carry them off. The deflection of drops by an air current (4m per sec) which corresponds to normal inhalation air was also tested with the aid of various glass tubes. Depending on the angle between the axis of the conical sprayer and the glass tube, drops of varying sizes were deflected. If we apply this to humans, it follows that larger drops can be inhaled only if the force of a cough reaches the open mouth directly or the nostrils from below. Drops over 100 u, due to their size and weight, should not penetrate beyond the front part of the nasal cavity or the back part of the pharyngeal wall. The filtration of drops through glass tubes was more marked if the stream contained more hinderances. Greater air velocity showed increased retentiveness on the part of the text tubes, most likely caused by the stronger explusion of drops.

In applying these conclusions to humans, the fact should be considered that the hinderances to the upper respiratory tract are without doubt larger than those in the glass tubes. In addition, the preliminary tests intentionally eliminated a number of instances which would make the passage of drops through the glass tubes difficult. For example, the spray was directed straight to the opening, and the axis of the sprayer came together with the axis of the glass tube at the initial points. Finally, only those figures may be used which were obtained using the velocity that corresponds to normal breathing. Since drops up to 20u passed even the finest tubes, it may be assumed that cough drops of similar size have the capacity to penetrate right into the lungs.

Following these preliminary tests, experiments were conducted on guinea pigs. Lange and Keschischian wanted to determine the number of inhaled germs that penetrate into the lungs and the number retained in the upper respiratory tract. The apparatus used initially was the arrangement described above, consisting of a glass box and a Buchner sprayer.

The animals were placed in the glass box so that the spray or the dust could not reach them directly. After the completion of the experiment, they were put to death with chloroform. The mucous membrane of the nasal and mouth cavity (and tongue) and the trachea, were carefully removed. Each mucous membrane was separately cut into pieces with scissors, mashed in a sterile mortar, and flooded with 5cc of NaCl solution. The upper section of the lung was separated from the lower section, they were cut into small pieces,

each mixed with 10cc, and mashed as evenly as possible. The lungs had been freed from the bronchi branches whenever it had been possible. The substances were poured out on separate agar plates, kept for three days at 37°, and then the germs were counted.

Floats of anthrax and subtilis spores or mixtures of dry spore substances and talcum were used in the spraying.

As regards the upper respiratory tract, this method renders only partially useful results. The figures obtained represent minimal values. Nevertheless, it was not unreasonable to expect that if the retention of germs showed considerable differences between moist and dry atomizing, they would have to be brought out by this method as well.

Following a large number of experiments, Lange and Keschischian determined how many of the inhaled germs (that is, germs found in the mucous membrane of the upper respiratory tract, the mouth cavity, and the lungs) on the average were to be found in the lung alone. Both moist and dry atomizing showed 50 per cent. This figure is most likely too high, for the bacilli settling in the upper respiratory tract were included only in part. It is certain, however, that the inhalation of even the smallest amounts of germs permits a relatively large portion of these to penetrate into the lungs, without any essential differences between drops and dust. The germ content of the individual lung sections usually varied, yet the differences were never regular enough to be considered in favor of a particular section. The fact that the germ quantities obtained by mashing the lungs did not come from the bronchi is certain because the hilus cuts did not contain more germs than the lung sections.

In another series of experiments, the method was improved by placing the animals in the inhalation tower (cf Findel and Kohlisch).

Air containing germ drops and dust was again channeled into the inhalation chamber described above. The spray or dust was produced by 20 to 200 strokes of a bicycle pump, distributed at 20- to 30-minute intervals. During that time, one or two guinea pigs inhaled the air inside the tower. Findel had fixed them in a cage so that only their heads were in the tower. Their nostrils were about 25 cm over the spray mist and the dust on the bottom of the tower. Since all larger drop and dust particles had settled on

the angles of the glass tube and on the bottom of the tower,
only a mist composed of the very smallest drop rose upwards
to the animals. A glass tube 8 mm in diameter was located
opposite the heads of the animals. This tube sucked the
air from the tower by means of a water pump throughout the
experiment, and channeled it through an Erlmeyer flask con-
taining 30 cc NaCl solution. One liter of air per minute
passed through this arrangement in all experiments.

Colical bacteria and hay bacillus spores were used
in the spraying, a mixture of dry hay bacilli and talcum in
the atomising. The breathing volume of the guinea pigs
(300 cc) was used as the basis (for the sake of simplicity
1/3 liter) to calculate the entire amount of germs inhaled.

After completion of the experiment, the animals were
put to death chloroform. The lungs removed mashed in mor-
tar, and the tissue float set up in cultures. The same
method was used to determine the germ content of the wash-
water, including the NaCl solution used to rinse the test
tubes and the rinse from the cotton wool filter.

Some of the variations in figures may be explained
by uneven breathing on the part on animals of similar size,
as well as by the fact that animals do not breathe calmly
and evenly, but react to the inhalation of dust with resis-
tance coming from the respiratory organs (panting, super-
ficial breathing), even though they seem to be sitting
quietly in their cages. In addition, the degree of dryness
plays an important part in dust experiments. It is a known
fact that even a slight rise in moisture leads to lumps of
just particles, diminishing their suspension capacity.

The ratio of germs which penetrated into the lungs
to total germs inhaled with moist atomizing was 7:30% (ave-
rage 21.5%), in dry atomizing 3:25% (average 12%). This
shows that with the same number of germs inhaled, those at-
tached to drops reach the lungs in amounts twice as large
as those attached to dust. Similar results become apparent
in test tube experiments as well. These experiments, how-
ever, should not be used to draw the conclusion that under
natural infectious conditions twice as many germs reach
the lungs with cough drops as with dust, because cough drops
are almost exclusively larger than those in the spray.

Experiments where guinea pigs were coughed at by con-
sumptive patients were expected to clarify the question
whether the inhalation of coughed-up drops and their aspira-
tion constituted a factor under natural conditions of infec-
tion. While a number of earlier scientists succeeded in

having guinea pigs infected by placing them in a box and having them coughed at through a tube, the matter became more complicated when, in an attempt to reproduce natural infection conditions of humans, the animals were free in a room opposite a coughing patient. The poorer results obtained when the animals were exposed in free space may have been caused by air currents which immediately swept the smallest drops away from the breathing space of the animals. This instance, however, cannot be of decisive importance. Lange and Keschischian had also obtained bad results when the animals were left loose, in spite of the fact that the experiments took place in a small, air-tight room, with static atmosphere. The weak air currents used in atomizing should have directed the smallest drops toward the animal instead of deflecting them. The chief difference is more likely caused by the fact that the most infectious small drops have very little tractive force. Their movement in the original direction stops in a very short time, or at least they are slowed down considerably by the resistance of air. The weak inhalation stream can draw only those small particles into the lung which accidentally reach the immediate proximity of the nostrils. In the inhalation tower, on the other hand, even a weak air current carries the smallest drops directly past the head of the animal.

Since only the finest drops are used in the tower experiment, Lange and Kreschischian found only primary tuberculosis in the animals. The neck glands had changed only to the point indicative of a general infection. It should be considered that a guinea pig needs only one bacillus to produce primary lung infection, and that according to experience, the receptivity of a very young child is very close to that of a guinea pig. According to Lange and Keschischian, primary infection caused by the inhalation of cough drops is possible but rare, because contrary to Flugge, they believed that only the smallest cough drops, approximately 10 to 20 u in size, and only in exceptional cases those up to 100 u, are of any consequence.

How can the frequent occurrence of lung infection in children be explained? They can probably also be traced back to an aerogenous infection. For this reason it is necessary to determine to what degree the second aerogenous possibility of communication, the dust infection, is of consequence in this regard. Together with Nowosselsky (2), B. Lange studied this problem extensively. In one of the first experimental series, he let guinea pigs inhale tubercular sputum in free space. We intend to mention the method only briefly.

The atomizing substance was a mixture of talcum and dry tubercular bacilli (bovine culture G. A.), 1/10 mg tubercular bacilli to 1 g talcum. 100 mg of moist bacillic substance were dried in an open container in an incubator at 37° for 24 hours.

Air was blown by two strokes of a bicycle pump at an inerval of several minutes into a glass flask half-filled with the dust mixture. The dust raised forced itself through a rectangular glass tube into a large, carefully air-tightened space.

As mentioned in the previous work, four guinea pigs were placed on a pedestal 30 cm away from the opening of the tube, so that their nostrils had to be hit directly by the cloud of dust. This arrangement allowed larger particles, in addition to the small ones, to enter the breathing space of the animal. The experiment lasted 10 minutes. Some animals were put to death immediately, others lasted 10 minutes. Some animals were put to death immediately, others several weeks later. In both cases, an infection had been effected.

In the second series, the guinea pigs were exposed to bacillic dust in the inhalation tower in order to determine the minimum amount of bacilli necessary for an infection. The attempt was made first to determine the number of bacilli present in the inhalation air, and then the number of those that had actually penetrated into the lungs of the animals.

The above-mentioned method was used in channeling the dust, exposing the animals, and so forth. However, the NaCl solution containing all germs from the air which had been drawn from the tower during the experiment was injected to guinea pigs in graded quantities instead of being cultured. The tubercle bacilli came from a three-week-old bovine culture; they were dried for 24 hours at 37°, mashed in mortar, and added to a specific amount of talcum powder. The death of the animals occurred four to five weeks later.

Both experimental groups showed that a lung infection is easily achieved in guinea pigs through the inhalation of bacillic dust. The prerequisite is merely dry dust and a sufficient quantity of tubercles in the dust mixture. Neither should the bacilli be killed or badly damaged by unnatural drying procedures.

At first glance, the quantity of bacilli contained in one gram of talcum powder appears very large. However, as proved by the results of air suction, only a very small fraction passes with the dust into the air. The larger portion of bacilli clings to larger particles which settle on the ground in a very short time. The portion of large particles of dust is certainly considerable. The inhalation experiments which used bacillic floats in the spray required a relatively large dosage of bacilli per 1 cc liquid.

If we compare the artificial dust experiments with the spray experiments from the point of view of bacilli content in one gram or one cc, it is safe to state that the spraying of small amounts of bacilli is more effective than dry atomizing. This is no doubt connected with the fact that bacilli cannot be distributed as effectively in dry dust as in liquid; and in addition, a considerable number of bacilli perish during the drying process. This deficit of bacilli in dry dust is compensated for only in part by the larger number of bacilli contained in one milligram of dry culture substance.

In the experiments where the dust was developed in free space, larger particles came into action in addition to atomic dust. Accordingly, a dissection of exposed guinea pigs revealed not only primary lung tuberculosis, but also partial cervical gland tuberculosis, and in one case even primary mesenterial gland tuberculosis. Experiments in the inhalation tower showed that only a few isolated germs were necessary to produce primary sources in the lung. In one case, 12 bacilli started two primary flocks, in another case 22 bacilli initiated five. Subsequent experiments showed similar results. It must be concluded that an infection may be achieved in guinea pigs even if only a few isolated bacilli penetrate into the lungs. There are no facts to contradict the assumption that one bacillus of a highly virulent culture is capable of producing one lung tubercle. Contrary to Kohlisch, these findings placed the minimum effective dosage much lower, while Findel's figures for bacilli floats (26 to 50 bacilli) are much higher. These differences are no doubt connected with the mode of calculation. However, these experimental results are in full accord with Chausse's view, namely, that a single inhaled bacillus both in dust and in drops can cause an infection in the lungs.

It was previously pointed out that 21.5% of the drops in an artificial spray penetrate into the lungs. At the same time, it was pointed out that this figure should not be applied without reservation to drops in the cough. The

portion of cough drops which reaches the lungs is definite-
ly smaller, since even the smallest cough drops which have
been observed so far are larger than the spray drops in-
haled in the experiment. On the other hand, the figure in
dry atomizing (12%) may be used in dust inhalation under
natural circumstances, because dust of such fineness oc-
curs frequently in nature or in dwellings. Retentive differ-
ences in drop and dust inhalation may be connected primarily
with the size and density of the particles.

No explanation was found for the fact that tubercles
can pass through the lungs without causing changes. B. Lange
and Nowosselsky could not deny this possibility.

It has been proven several times that bacteria which
are inhaled by animals in the form of fine drops or dust
cause typical primary tuberculosis in the lung. At times,
however, this leads rapidly to a blood infection, and, if
the amount of bacteria inhaled is large, signs of a general
infection are discovered in animals put to death four to six
weeks later. If in accordance with natural conditions small
amounts of bacilli are used, four to six weeks after inhala-
tion the animals frequently show no other changes but those
in the lungs, bronchial glands, and perhaps tracheal glands.
One of the chief reasons for aerogenous infection is the
great reciptivity of the mucuous membrane of the lung as
opposed to the nasal cavity and the intestine. Several ba-
cilli suffice to start an infection in the lungs, while
large amounts of agents are necessary to achieve a similar
effect in the nasal cavity or the intestine.

Important parallels arise between inhalation tuber-
culosis in guinea pigs and the primary complex in children,
as described by Parrot, Kuss, Ghon, Ranke. We would like
to point out in particular the severity of bronchial gland
disease and the close connection between lung sources and
gland disease. The picture of the so-called primary complex
in the guinea pig manifests itself most clearly in cases
with one or a few lung sources. The tracheal gland is mark-
edly swollen and slimy. As the disease progresses and sev-
eral sources appear in the lung, the relation is no longer
as clear. There are no principal differences between chan-
ges in the lung caused by drops or dust. The fact that
sometimes particularly large flocks were observed following
the inhalation of dry, pulverized matter may be connected
with the isolated location of fine bacilli drops and the
fact that, in addition to these, small clusters of bacilli
may become effective in the dust. Lange and Nowoselsky dis-
covered that the infection sources are located more frequently

in the right lung than in the left. In comparison to the
upper and lower section, the right center section was par-
ticularly often diseased. There was no noticeable priority
in the upper or lower section. Primary sources were seldom
found in the apical portion of the upper section. The sour-
ces were subpleural almost throughtout, and were initially
grey with a yellow center. The consistency was obviously
firm. Even this brief outline indicates that the primary
sources caused by artificial inhalation in guinea pigs are
similar to those in humans.

In another series of experiments, B. Lange and W.
Nowosselsky (3) performed inhalation experiments with strep-
tococci, pneumococci, chicken cholera bacilli, erysipelas
bacilli, mice typhoid bacilli, and hay bacilli spores.

The apparatus was essentially similar to the one pre-
viously described. In a number of experiments, the mice
were not fixed in a cage, but sat freely in a glass box, so
that the body was exposed to the indirect spray. To deter-
mine the amount of germs inhaled into the lungs, a counting
of germs in liquid culture following dilution took place in
addition to the plate method.

They found that various septicaemia agents which are
highly effective when injected can by no means infect to the
same degree from the lung. Only mice typhoid and chicken
cholera bacilli even in very small amounts are almost regu-
larly capable of causing a fatal infection. Highly virulent
erysipelas bacilli infect only if several thousands of germs
are inhaled. In the case of streptococci and pneumococci,
the effect of even very large quantities of germs was ques-
tionable.

Consequently, an infection depends on the number of
germs inhaled, and above all on the natural resistance of
the individual animals. It was generally observed that
"animal" bacteria are far more virulent than the most viru-
lent "culture" bacteria. A greater effect of repeated in-
halation infections as opposed to a singular infection could
not be proved, at least in the case of pneumococci, strepto-
cocci, and erysipelas bacilli.

What happens to germs inhaled into the lungs? Since
microbes contained in the air are bound to penetrate into
the animal organism during the process of breathing, the
frequently-found sterility in the lungs of slaughtered ani-
mals must be caused by a highly efficient "self-purifying"
quality of the lungs. The germs must either be quickly

destroyed or eliminated from the lungs. Some other saprophytes and even pathogenic bacteria act in the same way.

The bacteriacide sets in rapidly, sometimes even at the moment the germs enter the lungs. Earlier scientists had already determined that such a disappearance of germs rests on assimilation in the lung tissue itself, rather than an elimination to the outside or into the bronchial glands.

A further criterion for the process of infections is the presence or absence of inhaled germs in the blood or in other organs at specific times following inhalation. The presence of pneumococci, streptococci, and erysipelas bacilli has never been proven in the blood; this was only sometimes the case with hay bacilli spores. Advanced stages of infection caused by chicken cholera and mice typhoid bacilli, however, always showed bacteria in the blood.

We shall discontinue at this point in an effort to avoid an extended historical discussion, and refer the reader to a final summary by B. Lange (4) in the handbook Die Tuberkulose (Tuberculosis), 1943, published by Brauning. In addition, we would like to point out the chapter "Pathology, Ways and Sources of Infections," by H. Selter and W. Blumberg in Handbuch fur pathogene Mikroorganismen (Handbook of Pathogenic Microbes), 1928. Of interest to us is the final sentence, "Whether it is a direct infection caused by coughed-up bacilli drops, or an indirect infection caused by dry and pulverized cough drops, the drops alone always remain the infection agents."

It should also be mentioned that in France, Trillat (1, 2, 3, 4, 5) dealt in great detail (1925-1938) with contagiousness through aerosols containing bacteria and the problem of aerogenous immunization.

Hedvall wrote a chapter on the sources and spreading of infections in a handbook on tuberculosis published by von Hein, Kleinschmidt, and Uehlinger in 1958. He does not, however, mention any new findings.

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